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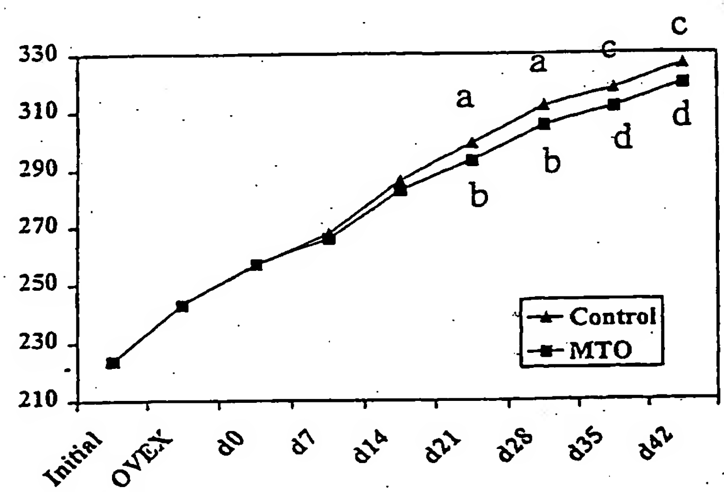
(54) Title: USE OF MODIFIED TALL OIL IN COMBINATION WITH DIETARY SUPPLEMENTS TO IMPROVE BODY COMPOSITION AND HEALTH STATUS



Values are means of 13 rats per dietary group.
[a,b]Significantly different at $P = .04$.
[c,d]Significantly different at $P \leq .07$.

(57) Abstract: New compositions for improving body compositions of humans or animals and methods of feeding those compositions to humans or animals are provided. The compositions comprise a mixture of a dietary supplement and MTO or CLA. Preferably the dietary supplement is an agent for altering the metabolism of a particular component in the human or animal (e.g., fat, protein, minerals, water, etc.). The compositions can be fed directly to the human or animal, mixed with a food or beverage to be consumed by the human or animal, or incorporated into a tablet or capsule. Consuming the compositions according to the invention results in reduced body weight, reduced body fat, and increased total body lean mass in the human or animal consuming the composition.

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USE OF MODIFIED TALL OIL IN COMBINATION WITH DIETARY SUPPLEMENTS TO IMPROVE BODY COMPOSITION AND HEALTH STATUS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention is broadly concerned with new compositions for improving the body composition of humans or animals and methods of using such compositions. More particularly, the compositions comprise a mixture of a dietary supplement (such as an agent for altering the metabolism of the human or animal) and a fatty acid source such as modified tall oil or conjugated linoleic acids.

Description of the Prior Art

Conjugated linoleic acid (CLA) was first identified by Ha et al., *Anticarcinogens From Fried Ground Beef: Heat Altered Derivatives of Linoleic Acid*, Carcinogenesis 8:1881-97 (1987), incorporated by reference herein. CLA is a collective term describing any of the positional and geometric conjugated dienoic isomers of linoleic acid (*cis* 9, *cis* 12-octadecadienoic acid). Linoleic acid (C18:2) has double bonds located at carbons 9 and 12 in the *cis* configuration. Conjugated linoleic acid has either the *cis* or *trans* configuration, or both, located on carbons 9 and 11, 10 and 12, or 11 and 13. It is thought that the *cis* 9, *trans* 11 form of CLA is the biologically active form which can be incorporated into phospholipids in the body.

Modified tall oil is derived from further processing of crude tall oil (a by-product from the kraft pulping of pine wood in the pulp and paper industry) and is a rich source (approximately 70% by weight) of CLA. Little information exists regarding the biological effects of MTO in animal species other than young, rapidly growing swine (see, e.g., U.S. Patent No. 6,020,377), incorporated by reference herein.

Postmenopausal women make up a significant portion (about 25%) of the population, with their numbers increasing each year. After menopause, women are highly susceptible to increased adiposity (particularly in the abdominal area) and an increased risk of osteoporosis. Estrogen replacement therapy can provide relief for many women who experience such menopausal symptoms and has, over the years, become increasingly popular. However, while estrogen therapy is successful in alleviating at least some problems commonly found in postmenopausal women, it is

not without side effects. In some cases, the side effects can be quite severe, including an increased risk of certain cancers (e.g., breast cancer). Estrogen has also been implicated in certain endometrial cancers.

Methods for treating osteoporosis have varied considerably but to date no totally satisfactory treatment is yet known. Another conventional treatment is to administer a calcium supplement to the patient. However, calcium supplementation by itself has not been successful in preventing or curing the disease.

There is a need for safe and effective treatments which reduce body weight gain, reduce adiposity, and increase lean content in mammals, particularly in humans.

SUMMARY OF THE INVENTION

The present invention overcomes these problems by broadly providing compositions and methods of using those compositions which improve the body compositions of the person or animal consuming the composition.

In more detail, the inventive methods comprise feeding to a human (males or females of any age) or animal (such as rats, poultry, cattle, horses, swine, dogs, cats, and fish) a composition comprising a mixture of a dietary supplement and a fatty acid source comprising conjugated linoleic acids (CLA) and/or modified tall oil (MTO).

As used herein, conjugated linoleic acid or CLA is intended to include any form of conjugated linoleic acid (i.e., any positional and/or geometrical conjugated dienoic isomer(s) of linoleic acid). Thus, any single isomer source, multiple isomer sources, and/or blended isomer sources are intended to be included within the terms "conjugated linoleic acid" or "CLA."

Also, as used herein, modified tall oil or MTO is intended to include any conjugated linoleic acid derivative of crude tall oil generated by the pulp in paper industry. Two particularly preferred formulations of MTO are set forth in Table A.

Table A. Chemical Makeup of Modified Tall Oil

Component	Broad Ranges % By Weight	Preferred Ranges % By Weight
Palmitic Acid, 16:0	up to about 5%	about 0.01 - 3%
Stearic Acid, 18:0	up to about 4%	about 0.01 - 3%
Oleic Acid, 18:1	up to about 25%	about 10-22%
Linoleic Acid, 18:2 (c9, c12)	up to about 10%	about 1.5-5.0%
Conjugated Linoleic Acid, 18:2 (c&t 9,11 mix)[a]	up to about 25%	about 15-22%
Conjugated Linoleic Acid, 18:2 (t9, t11)[b]	about 5 - 20%	about 10 - 17%
Conjugated Linoleic Acid, 18:2 (c10, c12)[c]	about 5-20%	about 8-16%
Conjugated Linoleic Acid, 18:2 (t10, c12)[d]	about 8-21%	about 9-18%
3 Conjugated Linoleic Acid Peaks, 18:2[e]	about 4 - 11%	about 6 - 10%
Unidentified CLA isomers	up to about 8%	about 4-6%
CLA 1 + CLA 2 Mixture	about 20-50%	about 30-40%
CLA 1 + CLA 2 + CLA 3 + CLA 4 + CLA 5 Mixture	about 60-85%	about 70-80%

[a] Hereinafter referred to as CLA 1.
[b] Hereinafter referred to as CLA 2.
[c] Hereinafter referred to as CLA 3.
[d] Hereinafter referred to as CLA 4.
[e] Hereinafter referred to as CLA 5.

As used herein, "c" and "t" refer to the *cis* and *trans* isomers of the particular conjugated linoleic acid. Furthermore, the number following "c" or "t" (such as c9 or t11) refers to the carbon atom at which a double bond is located. The numbers "18:2" refer to the number of carbon atoms and the number of double bonds in the acid, respectively.

Preferably, the dietary supplement is an agent which alters the metabolism of the human or animal. Even more preferably, the supplement is an agent which alters the metabolism by the human or animal of a component selected from the group consisting of fat, water, minerals (ash), protein, and mixtures thereof. Examples of such agents include sources of carnitine, chromium, creatine, anabolic agents (e.g., androstenedione), co-enzyme Q10, TCA intermediates (e.g., pyruvate, citrate, fumerate, and succinate), lipoid acid, betaine, beta-agonists, somatatropins, heavy metals (e.g., vanadium), botanical herbs (e.g., ginko, ephydryne), ATP, NADH,

hydroxymethyl butyrate, and mixtures thereof. As used herein, "sources" is intended to include any vehicle (e.g., pure form, salt form, as part of another product, etc.) by which the particular dietary supplement may be provided. For example, the phrase "sources of carnitine" is intended to include carnitine salts as well as carnitine-containing products. Furthermore, the source should be provided in a form which is digestible by the animal or human (e.g., chromium picolinate which is a digestible form of chromium).

The fatty acid source is present in the composition at a level of from about 0.01-99% by weight, preferably from about 0.01-50% by weight, and more preferably from about 0.25-20% by weight, based upon the total weight of the composition taken as 100% by weight. The supplement should be present in the composition at a level of from about 25 ppb to about 99% by weight, preferably from about 25 ppb to about 50% by weight, and more preferably from about 50 ppb to about 30% by weight, based upon the total weight of the composition taken as 100% by weight. The remainder of the composition should comprise a suitable carrier.

Advantageously, the compositions can be fed directly to the human or animal, mixed with a food or beverage to be consumed by the human or animal, coated on the surface of a food to be consumed by the human or animal, or incorporated into a tablet or capsule. When mixed with a food or beverage or coated on a food, the composition should be present in the food or beverage at a level of at least about 0.01% by weight, and preferably from about 0.25-30% by weight. Thus, the fatty acid source and the dietary supplement should be present in the concentrations set forth in Tables B and C, respectively.

Table B - Fatty Acid Source Concentrations

		Broad Range	Preferred Range
Meal Replacement		about 0.01 to 10%[a]	about 0.25 to 5%
Drink Mix			
	Liquid	about 0.01 to 15%	about 0.01 to 5%
	Dry Conc.	about 0.01 to 99%	about 0.5 to 50%
Bar		about 0.01 to 30%	about 0.25 to 20%
Tablet		about 0.01 to 99%	about 0.5 to 50%
Feed		about 0.01 to 10%	about 0.25 to 5%

[a] Percentages by weight are based upon the total weight of the food or beverage taken as 100% by weight.

Table C - Dietary Supplement Concentrations

		Supplement Range	Preferred Range
Meal Replacement		about 25 ppb to 10%[a]	about 50 ppb to 5%
Drink Mix			
	Liquid	about 25 ppb to 15%	about 50 ppb to 5%
	Dry Conc.	about 25 ppb to 99%	about 50 ppb to 50%
Bar		about 25 ppb to 30%	about 50 ppb to 20%
Tablet		about 25 ppb to 99%	about 50 ppb to 50%
Feed		about 25 ppb to 10%	about 50 ppb to 5%

[a] Percentages by weight are based upon the total weight of the food or beverage taken as 100% by weight.

The step of feeding the composition to a human or animal preferably comprises feeding sufficient quantities of the composition to reduce the body weight of the human or animal by at least about 1%, and preferably from about 2-5% after about 42 days when compared to the initial body weight of the human or animal, and to achieve a total body fat loss in the human or animal of at least about 1% and preferably from about 2-25% as compared to the initial body fat in the human or animal. Consuming the inventive composition also preferably results in a total body lean mass which is at least about 0.5% greater, and preferably from about 1-10% greater, after about 42 days than the initial body lean mass of the human or animal consuming the composition.

Furthermore, feeding the composition to an animal results in an increased bone density, and increased mineral, energy, and protein utilization. Thus, an animal being fed the composition for about 42 days will have an ash content which is at least about 1%, and preferably from about 2-25% greater than would be achieved by an otherwise identical feeding method free of the composition.

It will be appreciated that the improved body compositions properties can be very beneficial to the animal or human, particularly when the human is a post-menopausal woman. Furthermore, treating the human or animal according to the invention does not entail side effects present in most prior art methods.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph depicting the body weight gain over time of ovariectomized rats consuming diets containing MTO as compared to a control.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

EXAMPLES

The following examples set forth preferred methods in accordance with the invention. It is to be understood, however, that these examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

MATERIALS AND METHODS

Animals and Diets

1. Experiment 1

Twenty-six female Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with an initial weight of 224±6 g were individually placed in plastic cages with stainless steel wire bottoms in a windowless room maintained at 24-26°C and 70% relative humidity (RH). A 12-hour light/dark cycle with the light period being from 9:15 a.m. to 9:15 p.m. was maintained. The rats were housed in an animal care facility at Kansas State University, approved by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and all experimental procedures were approved by the Kansas State University Animal Care and Use Committee (Protocol No. 1531).

Upon arrival, rats were given free access to a nutritionally adequate diet and deionized water until assigned to a dietary treatment 12 days later. The diet, set forth in Table 1, was formulated by Dyets, Inc. (Bethlehem, PA) according to the AIN-93 recommendations described by Reeves, et al., *AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet*, J. Nutr. 123:1939-51 (1993), incorporated by reference herein. The deionized water was obtained from a water purification system (Millipore Corp., Marlboro, MA) and delivered through a stainless steel nipple watering system. At 5 days, rats weighing 243±11 g were ovariectomized under halothane anesthesia, and allowed 7 days to recover.

Table 1. Composition of basal diets

	Basal diets, g of ingredient/kg of diet	
Ingredient	Exp. 1[1]	Exp. 2[2]
Egg white	200	200
Corn starch	396.486	396.486
Dextrinized corn starch	132	132
Dextrose	100	100
α-tocopherol stripped soybean oil	70.014	----
Soybean oil (not stripped)	----	70.014
Cellulose	50	50
Mineral mix[3]	35	35
Vitamin mix	10	10
Biotin (1 mg/g biotin sucrose mix)	4	4
Choline bitartrate	2.5	2.5

[1] The experimental diets were created by adding either 1% α-tocopherol stripped soybean oil or 1% MTO to the basal diet. These oil additions were balanced in total fatty acid profile (see Tables 2 and 3).

[2] The experimental diets were created by adding either 1% soybean oil (not stripped), MTO, or CLA to the basal diet and by substituting L-carnitine (150 ppm), chromium picolinate (200 ppb), and/or creatine monohydrate (0.75%) at the expense of the complete mixed basal diet.

[3] As purchased, the mineral mix was zinc-free. Zinc carbonate was added to the diet to achieve a zinc level of 32 mg/kg.

During this recovery time, rats were trained to meal feed according to methods described by Noh et al., *The Lymphatic Absorption of Lipids is Normalized by Enteral Phosphatidylcholine Infusion in Ovariectomized Rats with Estrogen Replacement*, J. Nutr. Biochem. 8:152-61 (1997), incorporated by reference herein. Briefly, rats were fed 90% of the average free access intake, which was determined by averaging the intake of the prior 5 days. This equated to a daily feed intake during

the experiment of 15 g that was fed in meals of 6 g at 9:00 a.m. and 9 g at 4:00 p.m. Twelve days after arrival, rats weighing 257±9 g were assigned by body weight to one of two dietary treatment groups and fed their respective diets (see Table 1) for the remainder of the 42-day study.

Analyses of the MTO utilized in this experiment were carried out as described by *Official Methods of Analysis*, Association of Official Analytical Chemists, Arlington, VA, 16th Ed. (1995), incorporated by reference herein. These analyses showed that the MTO contained 70.44% conjugated linoleic acid as well as the other fatty acids set forth in Table 2. Over 90% of the CLA isomers in the MTO was comprised of the following four isomers: *cis* 9, *trans* 11 (34.46%); *trans* 10, *cis* 12 (25.27%); *cis* 10, *cis* 12 (16.38%); and *trans* 9, *trans* 11 (14.25%). Because the oil mixes (1% dietary inclusion) used to create the experimental diets contained the fatty acids necessary to equalize the diets in fatty acid profiles, the diet with MTO contained 0.5544% actual conjugated linoleic acid isomers.

Experimental diets were formulated by adding 1% of either an α-tocopherol stripped soybean oil mix or an MTO mix, both being matched in fatty acid profiles (see Table 3), to the standard basal diet. Thus, because of the pair feeding and matching of fatty acid profiles, any observed biological responses could be attributed to the conjugated isomers of linoleic acid found in MTO. The supplemental linoleic acid present in the diet containing soybean oil was *cis* 9, *cis* 12 linoleic acid, and the supplemental linoleic acid present in the diet containing MTO was predominantly conjugated linoleic acid. Experimental diets were mixed bi-weekly and stored in sealed and air-evacuated plastic containers in the dark at 6°C to maintain freshness.

Table 2. Analyzed composition of modified tall oil (Exp. 1)

Fatty acid	%
C16:0, palmitic acid	0.66
C16:1, palmitoleic acid	0.61
C18:0, stearic acid	----
C18:1, oleic acid[1]	23.46
C18:2, linoleic acid	4.83
C18:2, conjugated linoleic acid	70.44
Total	100.00

Isomeric profile of CLA

CLA isomer	% of total CLA
cis 9, *trans* 11	34.46
trans 9, *trans* 11	14.25
cis 10, *cis* 12	16.38
trans 10, *cis* 12	25.27
3 unidentified CLA isomers	9.64
Total	100.00

[1] Includes less than 2% C18:1, elaidic acid.

Table 3. Fatty acid composition of supplemental oil mixes (Exp. 1)[1]

Item	Soybean oil mix, g/100 g	Modified tall oil mix, g/100 g
Modified tall oil	----	78.70
α-tocopherol-stripped soybean oil	79.84	----
C16:0, palmitic acid	----	9.0
C18:0, stearic acid	----	4.5
C18:1, oleic acid	2.98	----
C18:2, linoleic acid	17.18	----
C18:3, linolenic acid	----	7.8

[1] The additions of these fatty acids made both oil mixes equal in fatty acid profile. The additions were made on the basis of the analyzed fatty acid profile of the MTO used in this study (Table 2). Pure α-tocopherol was added to both oil mixes at the rate of 6.9 mg/100 g of oil mixture.

2. *Experiment 2*

Thirty female Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with an initial weight of 200.3±6 g and 30 intact male Sprague-Dawley rats with an initial weight of 216.9±8 g were used in this experiment. Pre-trial care and handling of these rats were as described above in Experiment 1, except that the rats were allowed to consume the diets on an *ad libitum* basis. This study was also approved by the Kansas State University Institutional Animal Care and Use Committee (Protocol No. 1695.2).

At 5 days, female rats weighing 220.7±6 g were ovariectomized under halothane anesthesia, and allowed 7 days to recover. Twelve days after arrival, rats (females: 237.4±10 g; males: 283.2±11 g) were assigned by body weight to one of seven dietary treatment groups and fed their respective diets (as shown in Table 1) for the remainder of the 35-day study. The MTO used in this experiment was supplied by Hercules, Inc.; the chromium picolinate (CrPic) was purchased from Prince Agri. Products, Inc.; the creatine monohydrate (CMH) was pharmaceutical grade (CREAPURE™; 99% pure creatine, available from General Nutrition Center); the L-carnitine was supplied by Lonza, Inc.; and the CLA (Clareen™) was purchased from Conlinco, Inc.

Determination of Growth Performance
and Blood and Tissue Sampling

1. *Experiment 1*

Individual rats were weighed weekly for the determination of gain and feed conversion efficiency. Initially (i.e., just prior to initiation of the study), and at four and six weeks, serum samples were obtained from the six heaviest rats per dietary group by placing a capillary tube into the orbital sinus as described by Riley, *Adaptation of Orbital Bleeding Technique to Rapid Serial Blood Studies*, Proc. Soc. Exp. Biol. Med., 104:751-54 (1960), incorporated by reference herein. Briefly, the rats were bled following an 18-hour fast, and the serum was allowed to clot for 1 hour before it was centrifuged for 30 minutes. Serum was stored at -20°C until it was analyzed for cholesterol, PL, and α-tocopherol (α-TP). Upon completion of the 42-day study, six randomly selected rats per dietary group were humanely sacrificed, and the following tissues were collected: heart; brain; liver; kidneys; retroperitoneal fat; and the gastrocnemius muscle and abdominal fat depots. These tissues were weighed and then stored in plastic vials in the dark at -70°C until analyzed for cholesterol, PL, and α-TP.

2. *Experiment 2*

Rats and feed cups were weighed weekly to determine body weight gain, average feed intake, and feed conversion efficiency. Feed was added at two- to three-day intervals to maintain freshness. No blood sampling was done in this experiment.

Determination of Body Composition

1. Experiment 1

At the end of the growth trial, randomly selected rats (n=6) were euthanized by an overdose of CO_2 prior to being scanned via DEXA (dual-energy x-ray absorptiometry) for the determination of body composition. The validity of using DEXA for body composition analysis has recently been verified for rats weighing over 200 g as discussed by Bertin et al., *Evaluation of Dual-Energy X-Ray Absorptiometry for Body-Composition Assessment in Rats*, J. Nutr. 128:1550-54 (1998), incorporated by reference herein. A Hologic QDR-1000 instrument (Hologic, Waltham, MA) was used to determine the bone mineral content, bone mineral density, and fat and lean contents of each rat.

2. Experiment 2

At the end of the growth trial (35 days), all sixty rats were euthanized by cervical dislocation following halothane overdose. Weights for the heart, liver, and all abdominal fat (excluding mesenteric) were obtained for each rat. Rats (including heart, liver, and abdominal fat) were then stored frozen (-11.5°C) in airtight plastic bags until analyzed for body composition. Frozen rats (including heart, liver, abdominal fat, skin, and hair) were dipped in liquid nitrogen and then homogenized in a Waring Blender also containing liquid nitrogen. Duplicate samples from each rat were used to determine crude protein (CP) (N x 6.25) using a LECO FP-2000 Protein/Nitrogen analyzer (LECO Corp., St. Joseph, MI). Duplicate samples from each rat were also used to determine moisture and fat content with a paired CEM Lab Wave 9000 microwave and CEM automatic extraction and solvent recovery system (CEM, Matthews, NC). Moisture and fat content were determined by weight difference, and methylene chloride was used as the solvent for fat extraction. Additionally, ash content was determined following ashing of the samples overnight at 600°C. Body composition values are expressed on an "as is" basis.

Determination of Serum and Tissue Lipids

1. Experiment 1

Serum total cholesterol levels were determined using a commercially available enzymatic diagnostic kit (Catalog No. 352-20, Sigma Chemical, St. Louis, MO). For the analysis, 25 μL of serum were used, and absorbance was read at 500 nm with a UV-1201 spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD). Values were determined by substituting absorbance readings into

a standard curve equation. Whole tissues were minced finely with razor blades. Tissue subsamples, ranging from 300 mg (heart, brain, and adipose tissues) to 500 mg (liver and kidneys), were used for lipid extraction following the procedure described by Folch et al., *A Simple Method for the Isolation and Purification of Total Lipids From Animal Tissues*, J. Biol. Chem. 226:497-509 (1957), incorporated by reference herein. Briefly, lipids were extracted from the tissues with a mixture of chloroform:methanol 2:1 (v/v) containing 10 mg of butylated hydroxytoluene (BHT; 10 mg per 100 mL of methanol). Total cholesterol levels of the tissues were determined colorimetrically from the lipid extracts using *o*-phthalaldehyde as described by Rudel et al., *Determination of Cholesterol Using o-Phthalaldehyde*, J. Lipid Res. 14:364-66 (1973), incorporated by reference herein. Serum and tissue phospholipid (PL) levels were colorimetrically determined as described by Raheja et al., *New Colorimetric Method for the Quantitative Estimation of Phospholipids Without Acid Digestion*, J. Lipid Res. 14:695-97 (1973), incorporated by reference herein. Serum PL levels were determined on 100 µL aliquots of the lipid extract, as prepared above.

Serum and tissue α-TP were determined according to the methods described by Zaspel et al., *Determination of Alpha-Tocopherol in Tissues and Plasma by High-Performance Liquid Chromatography*, Anal. Biochem. 130:146-50 (1983), incorporated by reference herein. Briefly, 80 µL of serum were placed in test tubes containing 150 mg of Na_2SO_4 and 1 mL of acetone. An internal standard (100 µL of α-TP acetate) was added to each tube to verify recovery. This mixture was then centrifuged (10 min. at 1360 x g), and the resulting supernatant was filtered through a microfilter membrane (0.45 µm polytetrafluoroethylene (PTFE), Alltech Associates, Inc., Deerfield, IL), dried under N_2, and redissolved in 150 µL of chloroform:methanol 1:3 (v/v) prior to injection (15 µL) into the high performance liquid chromatography (HPLC). The α-TP levels were determined using a reverse-phase HPLC column (Alltima C18, 5 µm, 4.6 x 150 mm, Alltech Associates, Inc.) and Beckman System Gold software (Beckman Instruments, Inc., Fullerton, CA) as described by Noh et al. (cited above) and by Kim et al., *Marginal Zinc Deficiency Lowers the Lymphatic Absorption of α-Tocopherol in Rats*, J. Nutr. 128:265-70 (1998), incorporated by reference herein. Methanol was used as the mobile phase and was propelled at 2 mL/min. Detection was monitored at 292 nm (Module 166, Beckman Instruments, Inc.). Tissue α-TP analyses were identical to those described for serum except 100 mg (brain and kidneys) to 200 mg (liver) of tissue were used for homogenization in acetone. Additionally, 400 µL of internal standard were added

and 600 μL of chloroform:methanol 1:3 (v/v) were used to redissolve the mixture. Under these conditions, serum and tissue α-TP were eluted at 4.05 min. The standard curve (peak area vs. ng of α-TP) was constructed by using α-TP standards. Concentrations of α-TP from 75 to 300 ng yielded a linear curve ® = 0.999).

2. *Experiment 2*

No tissue analyses were conducted in this experiment.

Statistics

1. *Experiment 1*

Values for the data are presented as means ±SD. Statistical analyses for body composition and serum and tissue measurements were performed using paired *t* tests. Final body weight was used as a covariate in the analysis of organ weights (see, SAS User's Guide: Release 6.03, SAS Institute, Cary, NC). Differences were considered significant at $P \leq 0.05$.

2. *Experiment 2*

Data were analyzed by one-way ANOVA. Means were statistically separated using the LS Means function of SAS. The data were analyzed separately for males and females, and no covariates were used in the statistical analysis.

RESULTS

Body and Organ Weights

1. *Experiment 1*

All rats were observed to be healthy for the duration of this study. All meals were consumed within 1 hour of feeding. Rats fed the diet containing MTO had small reductions in average daily gain (ADG) during each of the weekly time intervals. This translated into a significant reduction in both growth parameters when determined for the duration of the trial (days 0-42). Similarly, weekly body weights were not affected by diet until week 3. Body weights (see Fig. 1) of rats fed the diet containing MTO were reduced in week 3, and this trend continued for the duration of the trial.

Weights of the heart, liver, and kidneys were similar between the dietary groups (data not shown); however, rats fed the diet containing MTO had less retroperitoneal fat. As shown in Table 4, this reduction in retroperitoneal fat was about 35%.

Table 4. Body compositional analysis of rats fed MTO (Exp. 1)

Item	Dietary group	
	Control	MTO
Body weight, g	293.82±4.22	295.90±12.37
Bone mineral content (BMC), g	7.31±0.26	7.24±0.21
Bone mineral density, g/cm^2	0.137±0.003	0.136±0.002
Fat, g	54.02±2.22^a	43.05±3.93^b
Fat-free, g	232.50±6.09^a	245.72±14.50^b
Fat-free + BMC, g	239.80±6.13	252.97±14.59
Fat, %	18.40±0.96^a	14.57±1.72^b
Fat-free, %	79.12±1.00^a	82.96±1.75^b
Retroperitoneal fat, g*	2.39±0.26^a	1.56±0.29^b

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).
* Quantitatively determined on the rats not scanned via DEXA (n = 6).

2. *Experiment 2*

All rats were observed to be healthy for the duration of this experiment. Dietary treatment did not affect ending body weights of either male or female rats (see Tables 5 and 6) in the present experiment. Feed intake and amount of feed per unit of gain also were not affected by dietary treatment. Daily weight gain was not affected in male rats, but female rats fed MTO and L-carnitine gained less weight on a daily basis than did rats fed MTO, L-carnitine, CrPic, and CMH.

Table 5. Combinations of feed additives for ovariectomized female rats (Exp. 2).

ITEM	Dietary group: CONTROL	MTO	MTO + L-CARNITINE	MTO + CHROMIUM PICOLINATE	MTO + CREATINE MONOHYDRATE	CLA + ALL OTHERS	MTO + ALL OTHERS	SEM
Initial BW, g	239.1	238.5	238.3	239.4	239.3	238.5	239.2	5.319
Final BW, g	301.9	304.3	288.2	305.0	307.3	291.3	331.0	19.369
Heart weight, g	0.90^{a}	0.95^{ab}	0.89^{a}	0.90^{ab}	0.95^{ab}	0.94^{ab}	1.03^{b}	0.053
Liver weight, g	6.16^{a}	7.23^{ab}	6.64^{ab}	7.05^{ab}	7.75^{b}	6.49^{ab}	7.49^{b}	0.529
Total ADG, g	1.80^{ab}	2.01^{ab}	1.55^{a}	1.87^{ab}	1.94^{ab}	1.54^{ab}	2.63^{b}	0.465
Total ADFI, g	15.51	15.16	14.55	15.36	15.78	14.57	16.36	0.951
Total F/G, g/g	8.66	7.54	9.39	8.21	8.13	9.46	6.22	4.236
Abdominal fat, g	8.95^{a}	6.21^{b}	5.79^{b}	5.63^{b}	5.68^{b}	5.05^{b}	4.35^{b}	0.878
Abdominal fat, % change from control	0	-30.8	-35.3	-37.1	-36.5	-43.6	-51.4	----
BODY COMPOSITION[c]								
Crude protein, %[d]	22.21	22.07	21.38	22.28	21.65	22.05	21.69	0.499
Moisture, %	63.63^{a}	64.87^{ab}	64.58^{ab}	65.65^{b}	64.75^{ab}	64.64^{ab}	65.19^{ab}	0.783
Ash, %	3.08^{a}	3.48^{ab}	3.89^{b}	3.68^{ab}	3.72^{b}	4.05^{b}	3.66^{ab}	0.199
Ash, % change from control	0	+11.5	+20.8	+16.3	+17.2	+24.0	+15.9	----
Total fat, %	11.08^{a}	9.58^{ab}	10.15^{ab}	8.39^{b}	9.88^{ab}	9.26^{ab}	9.46^{ab}	0.783
Total fat, % change from control	0	-13.5	-8.4	-24.3	-10.8	-16.4	-14.6	----

Values are means of four or five (MTO and MTO+L-carnitine) rats per dietary group.

[a,b] Means on a row with different superscripts differ ($P < .10$).

[c] Body composition values represent the means of duplicate analysis from each rat per dietary group.

[d] N x 6.25.

Table 6. Combinations of feed additives for intact male rats (Exp. 2).

ITEM	Dietary group							
	CONTROL	MTO	MTO + L-CARNITINE	MTO + CHROMIUM PICOLINATE	MTO + CREATINE MONOHYDRATE	CLA + ALL OTHERS	MTO + ALL OTHERS	SEM
Initial BW, g	283.4	285.8	281.9	284.2	284.2	287.7	284.7	5.396
Final BW, g	371.9	371.5	360.1	365.7	373.7	372.5	373.3	9.277
Heart weight, g	1.07	1.10	1.07	1.11	1.10	1.11	1.10	0.040
Liver weight, g	8.51^{ab}	9.20^{a}	8.37^{b}	9.01^{ab}	8.64^{ab}	8.41^{ab}	8.39^{ab}	0.380
Total ADG, g	2.53	2.45	2.46	2.33	2.56	2.43	2.53	0.168
Total ADFI, g	17.49	16.90	17.60	16.92	16.92	17.36	17.68	0.602
Total F/G, g/g	6.91	6.90	7.15	7.26	6.61	7.14	6.99	0.348
Abdominal fat, g	7.63^{a}	6.31^{ab}	5.81^{b}	6.32^{ab}	6.80^{ab}	7.00^{ab}	5.87^{b}	0.899
Abdominal fat, % change from control	0	-17.3	-23.9	-17.2	-10.9	-8.3	-23.1	----
BODY COMPOSITION[d]								
Crude protein, %[e]	23.08^{abc}	23.85^{a}	23.31^{ab}	23.11^{abc}	22.19^{bc}	22.36^{bc}	22.05^{c}	0.492
Moisture, %	66.15^{a}	67.01^{ab}	67.51^{ab}	66.43^{a}	67.76^{ab}	66.45^{a}	68.74^{b}	0.767
Ash, %	3.56	3.53	3.75	3.46	3.47	3.68	3.39	0.229
Total fat, %	7.21^{bc}	5.61^{ab}	5.43^{b}	7.00^{abc}	6.58^{abc}	7.51^{c}	5.82^{abc}	0.769
Total fat, % change from control	0	-22.2	-24.7	-2.9	-8.7	+4.0	-19.3	----

Values are means of four or five (MTO and MTO+L-carnitine) rats per dietary group.

[a,b,c] Means on a row with different superscripts differ ($P < .10$).

[d] Body composition values represent the means of duplicate analysis from each rat per dietary group.

[e] N x 6.25.

Body Composition Measurements

1. Experiment 1

DEXA scans of the rats revealed no differences in bone mineral content, bone mineral density, or in the combination of bone mineral content plus fat-free mass (Table 4). However, rats fed the diet containing MTO had significantly reduced fat (expressed either as total grams or as a percentage) and increased fat-free (expressed either as total grams or as a percentage). The percentage decrease in total body fat was about 21% and the percentage increase in fat-free was about 5% for rats fed the diet containing MTO.

2. Experiment 2

Feeding MTO or CLA in combination with the other additives had profound effects in terms of lowering abdominal fat and total body fat. The reductions in total body fat were offset by increases in total body water and total body ash content (Tables 5 and 6). In the ovariectomized females, reductions in abdominal fat ranged from 30.8% (MTO alone) up to 51.4% (MTO, L-carnitine, CrPic, and CMH) with the other dietary groups being intermediate. Interestingly, the responses to abdominal fat (Table 5) indicate an additive response to feeding L-carnitine, CMH, and/or CrPic in combination with MTO or CLA. Similar reductions were observed for total body fat. Increases in total body ash content ranged from 11.5% (MTO alone) up to 24.0% (CLA, L-carnitine, CrPic, and CMH) with the other dietary groups being intermediate.

In the intact male rats (Table 6), the range in abdominal fat reduction was from 8.3% (CLA, L-carnitine, CrPic, and CMH) up to 23.9% (MTO and L-carnitine). The range for total body fat reduction was from 2.9% (MTO and CrPic) up to 24.7% (MTO and L-carnitine).

Serum and Tissue Lipids

1. Experiment 1

Total cholesterol contents were increased in the liver, kidneys, and abdominal and retroperitoneal fat depots from feeding the diet containing MTO (see Table 7). Cholesterol levels in the brain, heart, and gastrocnemius muscle were not affected by dietary treatment group. Feeding the diet containing MTO resulted in a trend toward lowered serum cholesterol levels by 6 weeks.

Table 7. Tissue and serum cholesterol levels of rats fed MTO (Exp. 1)

Item	Dietary group Control	MTO
Tissue, µmol/g		
Liver	7.13±0.75[a]	7.85±0.66[b]
Heart	2.92±0.76	3.04±0.51
Brain	35.49±3.48	36.66±3.00
Kidneys	9.21±0.89[a]	10.56±0.89[b]
Gastrocnemius muscle	2.57±0.57	2.87±0.44
Abdominal fat	2.34±0.46[a]	3.14±0.46[b]
Retroperitoneal fat	2.21±0.55[a]	3.61±0.62[b]
Serum, mM		
Initial	1.64±0.09	
4 weeks	1.65±0.16	1.60±0.07
6 weeks	1.68±0.13[a]	1.59±0.07[b]

n = 6; values not sharing a common superscript are significantly different ($P < 0.10$).

Phospholipid content was increased in the liver of rats fed the diet containing MTO (see Table 8), but other tissue and serum levels were not affected by dietary treatment groups. Similar to cholesterol, there was a trend toward lowered serum PL levels by 6 weeks for rats fed the diet containing MTO.

Table 8. Tissue and serum phospholipid levels of rats fed MTO (Exp. 1)

Item	Dietary group Control	MTO
Tissue, µmol/g		
Liver	33.71±2.13[a]	36.09±1.61[b]
Heart	28.40±4.37	26.37±2.14
Brain	51.74±1.93	52.14±2.69
Kidneys	30.15±1.95	30.29±1.75
Gastrocnemius muscle	20.48±2.11	20.20±1.10
Abdominal fat	1.52±0.34	1.26±0.78
Retroperitoneal fat	1.14±0.83	0.86±0.34
Serum, mM		
Initial	1.93±0.21	
4 weeks	1.45±0.18	1.44±0.15
6 weeks	1.42±0.13[a]	1.33±0.06[b]

n = 6; values not sharing a common superscript are significantly different ($P < 0.10$).

Levels of α-TP were not affected by dietary treatment group in the brain, liver, and kidneys (see Table 9). Feeding of the diet containing MTO resulted in a small decrease in α-TP levels in the heart, but this diet significantly reduced α-TP levels in the gastrocnemius muscle. However, feeding of the diet containing MTO

significantly increased the α-TP levels of both fat depots (134.11±14.55 nmol/g vs. 81.66±17.94 nmol/g and 128.51±10.48 nmol/g vs. 92.87±13.92 nmol/g for the MTO-supplemented and control diets in the abdominal and retroperitoneal fat depots, respectively). Additionally, serum α-TP levels were reduced at both 4 and 6 weeks from feeding the diet containing MTO.

Table 9. Tissue and serum α-tocopherol levels of rats fed MTO (Exp. 1)

	Dietary group	
Item	Control	MTO
Tissue, nmol/mg total lipid		
Liver	141.95±14.34	146.72±13.34
Heart	122.23±6.86	116.67±5.25
Brain	59.04±3.58	56.89±3.77
Kidneys	79.47±13.59	74.67±7.84
Gastrocnemius muscle	35.12±2.86^{a}	30.89±2.95^{b}
Abdominal fat	81.66±17.94^{a}	134.11±14.55^{b}
Retroperitoneal fat	92.87±13.92^{a}	128.51±10.48^{b}
Serum, μM		
Initial	19.06±4.86	
4 weeks	22.04±0.91^{a}	19.18±1.78^{b}
6 weeks	25.37±2.40^{a}	20.64±1.86^{b}

n = 6; values not sharing a common superscript are significantly different ($P < 0.05$).

CONCLUSIONS

These data demonstrate that feeding modified tall oil elicits beneficial biological responses in a rat model used to emulate post-menopausal women. Specifically, modified tall oil slowed body weight gain (thus reducing total body weight), reduced adiposity (abdominal and total body), and increased lean content. These data further demonstrate that modified tall oil alters the metabolism of α-tocopherol in a manner that concentrates it in the adipose tissues. Modified tall oil reduces serum cholesterol and phospholipid levels and beneficially alters the body and serum and tissue compositions in ovariectomized rats which are commonly used as a model for post-menopausal women. Additionally, feeding modified tall oil or conjugated linoleic acid in combination with other agents that alter the metabolism of fat, protein, water, and mineralization (ash) decreases abdominal fat, decreases total body fat, increases total body ash content, and increases total body water

content. These nutritional supplements include L-carnitine, creatine monohydrate, chromium picolinate, and similar supplements. Finally, these data also demonstrate the differences in the ability of MTO to alter performance or body composition as compared to CLA. The differences in performance or body composition observed from feeding MTO must be related to other conjugated fatty acids or compounds contained within MTO.

We Claim:

1. A method of improving a body compositional property of a human or animal comprising the step of administering to the human or animal a composition comprising a mixture of a dietary supplement and MTO.

2. The method of claim 1, wherein said dietary supplement is an agent for altering the metabolism of said human or animal.

3. The method of claim 2, wherein said agent alters the metabolism of a component selected from the group consisting of fat, water, minerals, protein, and mixtures thereof.

4. The method of claim 1, wherein said dietary supplement is selected from the group consisting of sources of carnitine, chromium, creatine, anabolic agents, co-enzyme Q10, TCA intermediates, lipoic acid, betaine, beta-agonists, somatatropins, heavy metals, botanical herbs, ATP, NADH, hydroxymethyl butyrate, and mixtures thereof.

5. The method of claim 1, wherein said feeding step comprises feeding said composition to a human.

6. The method of claim 5, wherein said feeding step comprises feeding said composition to a post-menopausal woman.

7. The method of claim 1, wherein said feeding step comprises feeding said composition to an animal selected from the group consisting of rats, swine, cattle poultry, horses, dogs, cats, and fish.

8. The method of claim 1, wherein said mixture further comprises a food or beverage for said human or animal.

9. The method of claim 1, wherein said MTO is present in said composition at a level of from about 0.01-99% by weight, based upon the total weight of the composition taken as 100% by weight.

10. The method of claim 1, wherein said supplement is present in said composition at a level of from about 25 ppb to about 99% by weight, based upon the total weight of the composition taken as 100% by weight.

11. The method of claim 1, wherein said human or animal has an initial body weight, and wherein said feeding step comprises feeding said composition in sufficient quantities to reduce the body weight of said human or animal by at least about 1% when compared to said initial body weight.

12. The method of claim 1, wherein said human or animal has an initial total body fat concentration and wherein said feeding step comprises feeding said composition in sufficient quantities to achieve a total body fat loss in said animal or human of at least about 1%.

13. The method of claim 1, wherein said human or animal has an initial body lean mass and wherein said feeding step comprises feeding said composition in sufficient quantities to achieve a total body lean mass which is at least about 0.5% greater than said initial body lean mass.

14. The method of claim 1, wherein said feeding step comprises feeding said composition to an animal in sufficient quantities to achieve an ash content which is at least about 1% greater than an otherwise identical feeding method free of said composition.

15. A composition comprising a mixture of a dietary supplement and MTO.

16. The composition of claim 15, wherein said dietary supplement is an agent for altering the metabolism of a human or animal.

17. The composition of claim 16, wherein said agent alters the metabolism of a component selected from the group consisting of fat, water, minerals, protein, and mixtures thereof.

18. The composition of claim 15, wherein said dietary supplement is selected from the group consisting of sources of carnitine, chromium, creatine monohydrate, androstenedione, carnitine, chromium, creatine, anabolic agents, co-enzyme Q10, TCA intermediates, lipoic acid, betaine, beta-agonists, somatatropins, heavy metals, botanical herbs, ATP, NADH, hydroxymethyl butyrate, and mixtures thereof.

19. The composition of claim 15, wherein said MTO is present in said composition at a level of from about 0.01-99% by weight, based upon the total weight of the composition taken as 100% by weight.

20. The composition of claim 15, wherein said supplement is present in said composition at a level of from about 25 ppb to about 99% by weight, based upon the total weight of the composition taken as 100% by weight.

21. A tablet or capsule including therein a quantity of a mixture of a dietary supplement and MTO.

22. The tablet or capsule of claim 21, wherein said dietary supplement is an agent for altering the metabolism of a human or animal.

23. The tablet or capsule of claim 22, wherein said agent alters the metabolism of a component selected from the group consisting of fat, water, minerals, protein, and mixtures thereof.

24. The tablet or capsule of claim 21, wherein said dietary supplement is selected from the group consisting of sources of carnitine, chromium, creatine monohydrate, androstenedione, carnitine, chromium, creatine, anabolic agents, co-enzyme Q10, TCA intermediates, lipoic acid, betaine, beta-agonists, somatatropins, heavy metals, botanical herbs, ATP, NADH, hydroxymethyl butyrate, and mixtures thereof.

25. The tablet or capsule of claim 21, wherein said MTO is present in said mixture at a level of from about 0.01-99% by weight, based upon the total weight of the mixture taken as 100% by weight.

26. The tablet or capsule of claim 21, wherein said supplement is present in said mixture at a level of from about 25 ppb to about 99% by weight, based upon the total weight of the mixture taken as 100% by weight.

27. The combination of:

a food or beverage for a human or animal; and

at least about 0.01% by weight of a composition comprising a dietary supplement and MTO, said percentage by weight being based upon the total weight of the food or beverage taken as 100% by weight.

28. The combination of claim 27, wherein said dietary supplement is an agent for altering the metabolism of the human or animal.

29. The combination of claim 28, wherein said agent alters the metabolism of a component selected from the group consisting of fat, water, minerals, protein, and mixtures thereof.

30. The combination of claim 27, wherein said dietary supplement is selected from the group consisting of sources of carnitine, chromium, creatine monohydrate, androstenedione, carnitine, chromium, creatine, anabolic agents, co-enzyme Q10, TCA intermediates, lipoic acid, betaine, beta-agonists, somatatropins, heavy metals, botanical herbs, ATP, NADH, hydroxymethyl butyrate, and mixtures thereof.

31. The combination of claim 27, said combination comprising a food presenting an outer surface and said composition being coated on the surface of said food.

32. The combination of claim 27, said composition being admixed with said food or beverage.

33. The combination of claim 27, wherein said MTO is present in said composition at a level of from about 0.01-99% by weight, based upon the total weight of the composition taken as 100% by weight.

34. The combination of claim 27, wherein said supplement is present in said composition at a level of from about 25 ppb to about 99% by weight, based upon the total weight of the composition taken as 100% by weight.

35. A method of improving a body compositional property of a human or animal comprising the step of administering to the human or animal a composition comprising a mixture of a dietary supplement and CLA.

36. The method of claim 35, wherein said dietary supplement is an agent for altering the metabolism of said human or animal.

37. The method of claim 36, wherein said agent alters the metabolism of a component selected from the group consisting of fat, water, minerals, protein, and mixtures thereof.

38. The method of claim 35, wherein said dietary supplement is selected from the group consisting of sources of carnitine, chromium, creatine, anabolic agents, co-enzyme Q10, TCA intermediates, lipoic acid, betaine, beta-agonists, somatatropins, heavy metals, botanical herbs, ATP, NADH, hydroxymethyl butyrate, and mixtures thereof.

39. The method of claim 35, wherein said feeding step comprises feeding said composition to a human.

40. The method of claim 39, wherein said feeding step comprises feeding said composition to a post-menopausal woman.

41. The method of claim 35, wherein said feeding step comprises feeding said composition to an animal selected from the group consisting of rats, swine, cattle poultry, horses, dogs, cats, and fish.

42. The method of claim 35, wherein said mixture further comprises a food or beverage for said human or animal.

43. The method of claim 35, wherein said CLA is present in said composition at a level of from about 0.01-99% by weight, based upon the total weight of the composition taken as 100% by weight.

44. The method of claim 35, wherein said supplement is present in said composition at a level of from about 25 ppb to about 99% by weight, based upon the total weight of the composition taken as 100% by weight.

45. The method of claim 35, wherein said human or animal has an initial body weight, and wherein said feeding step comprises feeding said composition in sufficient quantities to reduce the body weight of said human or animal by at least about 1% when compared to said initial body weight.

46. The method of claim 35, wherein said human or animal has an initial total body fat concentration and wherein said feeding step comprises feeding said composition in sufficient quantities to achieve a total body fat loss in said animal or human of at least about 1%.

47. The method of claim 35, wherein said human or animal has an initial body lean mass and wherein said feeding step comprises feeding said composition in sufficient quantities to achieve a total body lean mass which is at least about 0.5% greater than said initial body lean mass.

48. The method of claim 35, wherein said feeding step comprises feeding said composition to an animal in sufficient quantities to achieve an ash content which is at least about 1% greater than an otherwise identical feeding method free of said composition.

49. A composition comprising a mixture of a dietary supplement and CLA.

50. The composition of claim 49, wherein said dietary supplement is an agent for altering the metabolism of a human or animal.

51. The composition of claim 50, wherein said agent alters the metabolism of a component selected from the group consisting of fat, water, minerals, protein, and mixtures thereof.

52. The composition of claim 49, wherein said dietary supplement is selected from the group consisting of sources of carnitine, chromium, creatine monohydrate, androstenedione, carnitine, chromium, creatine, anabolic agents, co-enzyme Q10, TCA intermediates, lipoic acid, betaine, beta-agonists, somatatropins, heavy metals, botanical herbs, ATP, NADH, hydroxymethyl butyrate, and mixtures thereof.

53. The composition of claim 49, wherein said CLA is present in said composition at a level of from about 0.01-99% by weight, based upon the total weight of the composition taken as 100% by weight.

54. The composition of claim 49, wherein said supplement is present in said composition at a level of from about 25 ppb to about 99% by weight, based upon the total weight of the composition taken as 100% by weight.

55. A tablet or capsule including therein a quantity of a mixture of a dietary supplement and CLA.

56. The tablet or capsule of claim 55, wherein said dietary supplement is an agent for altering the metabolism of a human or animal.

57. The tablet or capsule of claim 56, wherein said agent alters the metabolism of a component selected from the group consisting of fat, water, minerals, protein, and mixtures thereof.

58. The tablet or capsule of claim 55, wherein said dietary supplement is selected from the group consisting of sources of carnitine, chromium, creatine monohydrate, androstenedione, carnitine, chromium, creatine, anabolic agents, co-enzyme Q10, TCA intermediates, lipoic acid, betaine, beta-agonists, somatatropins, heavy metals, botanical herbs, ATP, NADH, hydroxymethyl butyrate, and mixtures thereof.

59. The tablet or capsule of claim 55, wherein said CLA is present in said mixture at a level of from about 0.01-99% by weight, based upon the total weight of the mixture taken as 100% by weight.

60. The tablet or capsule of claim 55, wherein said supplement is present in said mixture at a level of from about 25 ppb to about 99% by weight, based upon the total weight of the mixture taken as 100% by weight.

61. The combination of:

a food or beverage for a human or animal; and

at least about 0.01% by weight of a composition comprising a dietary supplement and CLA, said percentage by weight being based upon the total weight of the food or beverage taken as 100% by weight.

62. The combination of claim 61, wherein said dietary supplement is an agent for altering the metabolism of the human or animal.

63. The combination of claim 62, wherein said agent alters the metabolism of a component selected from the group consisting of fat, water, minerals, protein, and mixtures thereof.

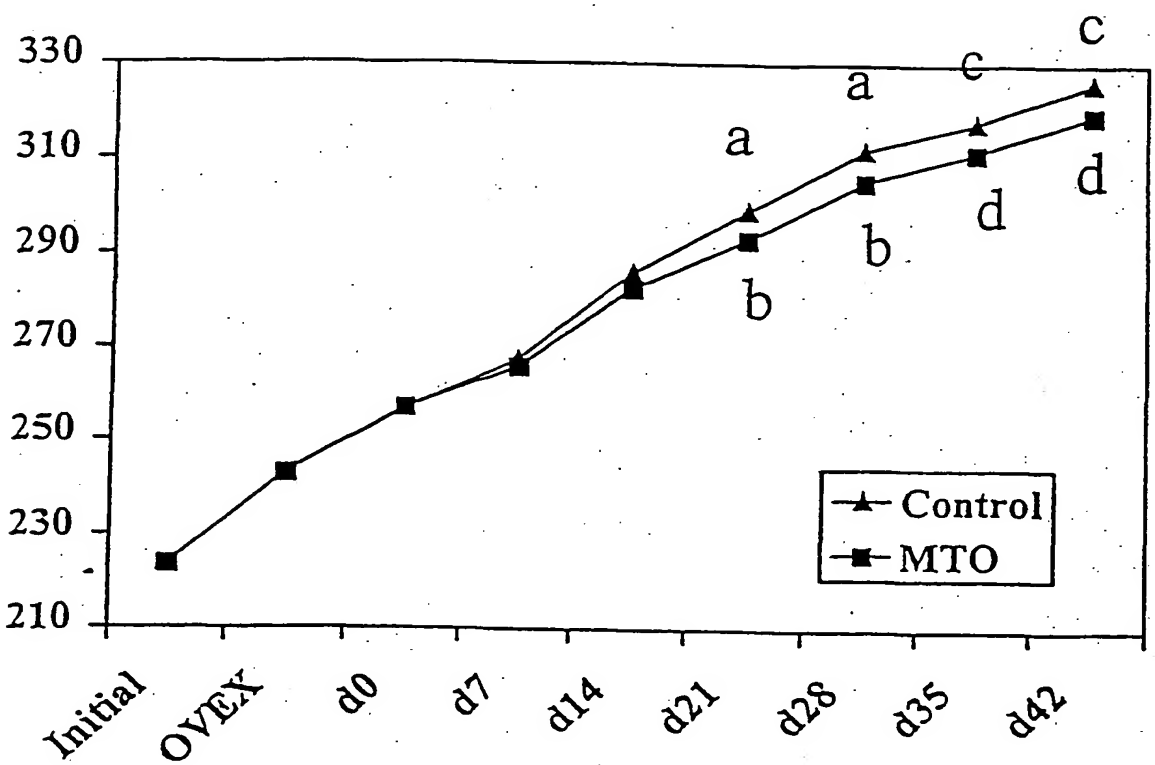
64. The combination of claim 61, wherein said dietary supplement is selected from the group consisting of sources of carnitine, chromium, creatine monohydrate, androstenedione, carnitine, chromium, creatine, anabolic agents, co-enzyme Q10, TCA intermediates, lipoic acid, betaine, beta-agonists, somatatropins, heavy metals, botanical herbs, ATP, NADH, hydroxymethyl butyrate, and mixtures thereof.

65. The combination of claim 61, said combination comprising a food presenting an outer surface and said composition being coated on the surface of said food.

66. The combination of claim 61, said composition being admixed with said food or beverage.

67. The combination of claim 61, wherein said CLA is present in said composition at a level of from about 0.01-99% by weight, based upon the total weight of the composition taken as 100% by weight.

68. The combination of claim 61, wherein said supplement is present in said composition at a level of from about 25 ppb to about 99% by weight, based upon the total weight of the composition taken as 100% by weight.



Values are means of 13 rats per dietary group.
[a,b]Significantly different at $P = .04$.
[c,d]Significantly different at $P \leq .07$.

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/21242

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/205, 35/78, 9/14, 9/20, 9/48, 9/50; A61P 3/04
US CL :514/556, 909; 424/196.1, 439, 451, 464, 489; 426/2, 442, 542
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/556, 909; 424/196.1, 439, 451, 464, 489; 426/2, 442, 542

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, USPATFULL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,020,377 A (O'QUINN ET AL) 02 FEBRUARY 2000 (02/02/00) see abstract, column 1, lines 6-8, column 2, lines 11-26, and Table A.	15-26
Y	US 5,124,357 A (NEWTON ET AL) 23 JUNE 1992 (23/06/92) see abstract.	15-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 SEPTEMBER 2001	20 NOV 2001

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	HELEN NGUYEN
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/21242

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-26

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.